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THE MACROMOLECULAR PARAMETERS OF ASCITES TUMOR CELL H-RNA

SUMMARY

A concerted analysis of sedimentation, light scattering and small-angle X-ray-scattering data on high-molecular-weight Ehrlich ascites tumor cell RNA has been carried out. The three sets of data have been found to be in quantitative agreement with a compact structure consisting of short double-helical rodlike subunits, which account for 90 % of the RNA mass.

INTRODUCTION

Recent developments in the theories of scattering of electromagnetic radiation[1,2] as well as of the hydrodynamics of macromolecules in solution[3–5] have greatly extended the possibility of quantitative comparison of results obtained by unrelated techniques and, thus, of the quantitative verification of conclusions about the structure of macromolecules reached from experiments that are based on different physical phenomena. Such an analysis has been carried out recently in the case of the globular protein, β-lactoglobulin, in which the thermodynamic light-scattering information[6] was used to predict hydrodynamic[7,8] (sedimentation) and electrokinetic[7] (electrophoresis) parameters, while geometric small-angle X-ray-scattering parameters[9] were used to verify quantitatively structural information inferred from the hydrodynamic[7] behavior of the molecules in various states of aggregation. It is the purpose of this paper to report on a similar analysis of structural data obtained from light scattering[10,11], small-angle X-ray scattering[12,13], sedimentation[14] and viscosity[14] with a large biological macromolecule, namely Ehrlich ascites tumor cell H-RNA. Specifically it will be shown how a detailed structural model deduced from small-angle X-ray-scattering data can be compared with the light-scattering radius of gyration as well as the sedimentation distribution and the intrinsic viscosity of the system. A similar comparison between light scattering and sedimentation data has been described by BERNARDI *et al.*[15], for the rigid-rod degradation product of DNA.

Abbreviation: H-RNA, high-molecular-weight RNA.

* Eastern Utilization Research and Development Division, Agricultural Research Service. U.S. Department of Agriculture.

EXPERIMENTAL INFORMATION

Ehrlich ascites tumor cell H-RNA was first prepared by COLTER AND BROWN[14], who showed it to have an intrinsic viscosity of 0.40 dl/g and to consist reproducibly of two ultracentrifugal components, 63 % of the material sedimenting with a sedimentation coefficient of 34 S and 37 % with an $s_{20,w}$ value of 18 S in a pH 7, 0.15 ionic strength medium. Light-scattering measurements[10,11] in the same medium gave a weight-average molecular weight, $\overline{M}_w$, of $1.4 \cdot 10^6$ and a Z-average radius of gyration of 335 ± 40 Å, indicating a compact structure. Small-angle X-ray-scattering examination of the same material[12,13] revealed an extended secondary structure in the same medium, as well as after dialysis against distilled water: 90 % of the mass of ascites tumor cell H-RNA consists of an array of rods with a radius of gyration about their axis, R_c, of 8.0 Å and an average length of approx. 100 Å. If a partial specific volume, $\bar{v}$, of 0.58 is used[44], the mass per unit length of the rods, M/L, can be calculated to be 210, which corresponds well to a structure such as a WATSON–CRICK double helix, with ribose in place of deoxyribose. Typical structures consistent with these results are represented schematically on Fig. 1, where I is a zig-zag chain of rods, II is a cross-linked compact array of the rods, III is a branched polymer type of structure.

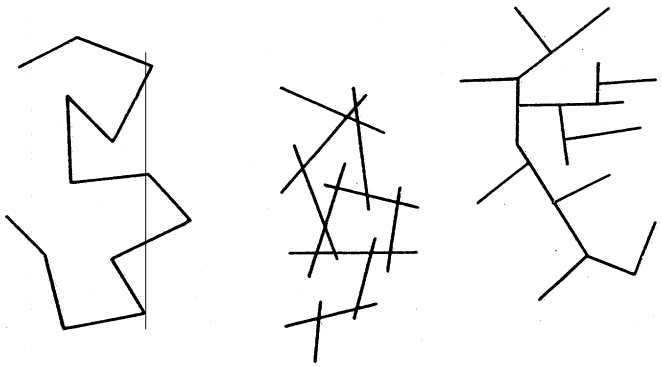

Fig. 1. Schematic models of H-RNA structure seen by small-angle X-ray scattering; I, zig-zag chain of rods; II, cross-linked polymer; III, branched polymer.

It was furthermore observed both in light scattering and small-angle X-ray-cattering experiments that, on standing, the superstructure of the H-RNA degraded. After several days, the breakdown product became stabilized at an angular distribution of the X-ray-scattering characteristic of short rigid rods having $R_c = 8.0$ Å. The molecular weight, M, and radius of gyration, R, of these rods can be obtained by plotting the logarithm of the small-angle X-ray-scattering intensity, $j_n(s)$, as a function of the square of the angular parameter, s, in the usual GUINIER plot[16,17].

STRUCTURAL ANALYSIS

On the basis of the small-angle X-ray-scattering data on the fresh and degraded RNA's, the structural model of H-RNA was taken as an array of short rods, such as the structures shown on Fig. 1, with the structural subunit rodlets having parameters identical with those of the degradation product ($M = 16400$; $l = 78$ Å; $r = 11.3$ Å; $\bar{v} = 0.58$). From this, the sedimentation and light-scattering parameters of the undegraded structure were calculated using the equations of HEARST AND STOCKMAYER[3], ZIMM AND KILB[4] and HERMANS AND HERMANS[1]. Since H-RNA is not a monodisperse system and for polydisperse systems these theories require a knowledge of various types of averages, a molecular weight distribution had to be obtained first. This was done by combining the equations of GIERER[20] and SUEOKA[21].

While a number of relations between the molecular weight and hydrodynamic parameters of macromolecules has been proposed[22], the first being that of SADRON[23], in the present case the equation of GIERER[20], developed specifically for H-RNA, was chosen as most appropriate. According to GIERER, the molecular weight of H-RNA is related to the sedimentation coefficient by

$$M = as^{2.2} \tag{4}$$

Then, for a polydisperse system, the weight-average molecular weight $\bar{M}_w = a\Sigma C_i s_i^{2.2}/\Sigma C_i$, where a is a constant depending only on the medium, and C_i and s_i are the concentrations and sedimentation coefficients of the sedimenting components i*. Using the bimodal distribution observed for this H-RNA and the light scattering $M_w = 1.4 \cdot 10^6$, a is found to have a value of 824, while the molecular weights of the 34-S and 18-S components come out at $1.9 \cdot 10^6$ and $5.0 \cdot 10^5$. The molecular weight distribution calculated by the method of SUEOKA[21] using the GIERER-type relation, $M = 824s^{2.2}$, (taking areas in increments of 1 S unit) is shown in Fig. 3. This distribution, independent of assumptions on molecular structure[20], presents a bimodal pattern, composed of two fairly sharp principal fractions. If, as a first approximation, the system is regarded as consisting of two components, the M_w/M_n values listed in

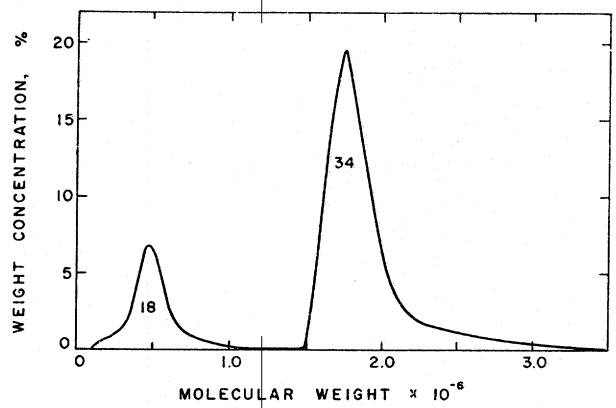

Fig. 3. Molecular weight distribution of Ehrlich ascites tumor cell H-RNA.

* This relation has been verified for various H-RNA's by a number of investigators[15,24,25].

where θ is the angle formed between the incident and scattered beams, λ is the wavelength of the radiation and $\varphi(s)$ is a residual function expressing the deviation of the scattering from an exponential relation at higher angles. The results obtained with the degradation product are shown in such a plot in Fig. 2. From the slope, the radius

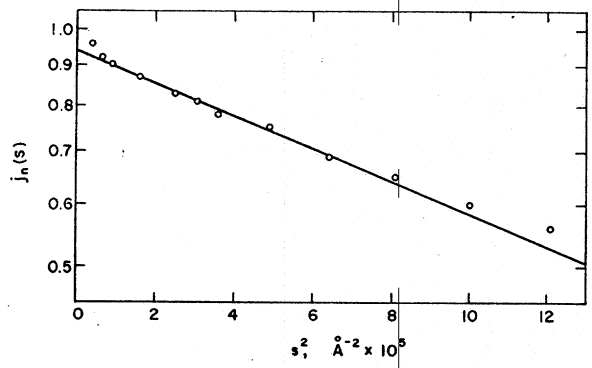

Fig. 2. Small-angle X-ray-scattering data of H-RNA degradation product: 18 days at 25°.

of gyration, averaged over all axes of rotation, R, is found to be 18.9 Å. Neglecting virial and multicomponent effects, the molecular weight, M, can be calculated from the intercept, $j_n(0)$ by[17,18]

$$M = \frac{N_A}{Q_s C_e (1 - \rho_0 \psi)^2} \left[2\sqrt{\frac{\pi}{3}}\, j_n(0) R - \frac{1}{\pi} \int_0^\infty s^{-2} \varphi(s) ds \right] \tag{2}$$

where N_A is Avogadro's number, ρ_0 is the electron density of the solvent (electrons·Å^{-3}), C_e is the concentration expressed as the ratio of the number of electrons in the solute to the total number of electrons in the solution, ψ is the electron partial specific volume of the solute (Å^3·electrons^{-1}) and Q_s is the ratio of the number of electrons to the mass of the molecule. Using this equation, the data of Fig. 2 result in a degradation product molecular weight of 16400, when a value of 0.58 is used for $\bar{v}$ ($\bar{v} = 10^{24}\, Q_s \psi$). Rigid rods of such molecular weight and $\bar{v}$ and a radius of 11.3 Å (which corresponds to $R_c = 8.0$ Å) have a length, l, of 78 Å. The radius of gyration, R, of a short rod, averaged over all three axes of coordinates, is given by

$$R^2 = \frac{1}{3} \left[\frac{r^2}{2} + 2 \left(\frac{r^2}{4} + \frac{l^2}{12} \right) \right] \tag{3}$$

Introducing the above values of r and l into Eqn. 3 results in a calculated R of 19.5 Å, which is in good agreement with the experimental value of 18.9 Å. Sedimentation experiments on the ascites tumor cell RNA breakdown product have shown it to sediment as a single sharp peak[13], with $s_{20,w} = 3.4$ S*.

* In recent experiments[19] on the degradation kinetics of similar preparations of RNA from rat liver, it was observed that $s_{20,w}$ decreases rapidly for the first 24 h, indicating a rapid decrease in molecular weight. This is accompanied, however, only by a slight release of alcohol-soluble low-molecular-weight product. After 3 days the molecular weight does not change any further, as indicated by the constancy of the attained value of $s_{20,w}$. In this respect these kinetics are similar to those reported by BERNARDI *et al.*[15] for the degradation of DNA.

for small-angle X-ray scattering. Since the information yielded by the two techniques is described by Eqn. 6, the only difference being in range of s covered, the two sets of data should form a continuous curve of intensity as a function of s.

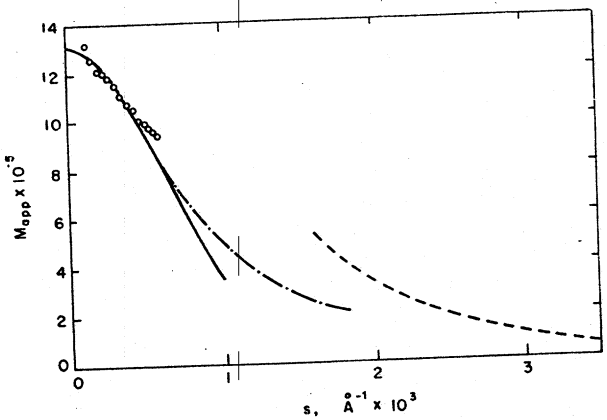

Fig. 4. Light scattering and small-angle X-ray scattering of H-RNA. Circles: light-scattering experimental points, 0.043 g/l; solid line: GUINIER plot fit of these points; dot–dash line: theoretical scattering from zig-zag chain (HERMANS–HERMANS); dashed line: small-angle X-ray-scattering data, at 16.1 g/l.

A simultaneous plot of the two sets of scattering data is shown in Fig. 4, where the intensity, expressed as apparent molecular weight, is plotted as a function of s^*. Theoretical scattering curves calculated for the Gaussian region (Eqn. 1) and for a zig-zag chain, according to the equation of HERMANS AND HERMANS[1], are plotted for comparison. As expected, the light-scattering points, obtained at a concentration of 0.043 g/l, follow closely the exponential relation in the region of lower angles. Above 100° they begin to deviate upward, as expected from Eqn. 1. The X-ray-scattering data fall above the curve calculated with the HERMANS–HERMANS equation, which is not surprising since in it the width of the rod is not taken into account. In the region of s below $3 \cdot 10^{-3}$ $Å^{-1}$, the X-ray data, obtained at a concentration of 16.1 g/l, rise gradually and display a general tendency toward the light-scattering data, reflecting the contribution to the small-angle X-ray scattering of the superstructure of the H-RNA which is the only feature seen by light scattering. Since the two sets of data are taken at finite concentration, and virial effects are neglected, they can be considered to be in reasonable agreement, confirming the mutual consistency of the two sets of measurements.

* In the case of light scattering this is simply $1/(K_c/R_\theta)$. In X-ray scattering this involves transformation of the experimental intensity, $j_n(s)$, measured for "infinite slit" collimation and reported previously[13], to the intensity, $i_n(s)$, for "point source" collimation. This is accomplished by numerical integration of the relation[16,17]:

Table I are obtained. The $\overline{M_w}/\overline{M_n}$ ratios of 1.13 and 1.02 for the light and heavy fractions, respectively, indicate little polydispersity in either and justify the validity of the above treatment in terms of the GIERER equation.

TABLE I

CALCULATED MOLECULAR PARAMETERS OF H-RNA

	18-S fraction	*34-S fraction*	*Total*
$\overline{M_w}$	$4.79 \cdot 10^5$	$1.88 \cdot 10^6$	$1.41 \cdot 10^6$
$\overline{M_n}$	$4.23 \cdot 10^5$	$1.84 \cdot 10^6$	$8.73 \cdot 10^5$
$\overline{M_w}/\overline{M_n}$	1.13	1.02	1.62
$(\overline{R^2_z})^{1/2}$ (Å)	177	343	329
$(\overline{R^2_n})^{1/2}$ (Å)	159	336	230
$(\overline{R^2_z})^{1/2}$ (Å) exptl.	—	—	335 ± 40

Calculation of light-scattering parameters

Taking for structural model the array of rods with the molecular parameters of the degradation product, the light-scattering radius of gyration distribution was calculated for Structure I of Fig. 1. HERMANS AND HERMANS[1] have shown that, for a zig-zag chain, the radius of gyration, R, is related to the length of the stiff rods, l, and their number, N, by

$$R = (1/\sqrt{6})\,(N - 1 + 1/2N)^{1/2} \tag{5}$$

Values of R were calculated according to this equation at increments of $N = 1$ over the entire molecular weight distribution of Fig. 3. The average of the light-scattering radius of gyration is

$$(\overline{R^2})^{1/2} = \left(\frac{\sum_i n_i M_i^2 R_i^2}{\sum_i n_i M_i^2} \right)^{1/2} \tag{6}$$

This quantity was evaluated and the results are summarized in Table I for each of the main fractions individually and for the entire RNA. The calculated overall average radius of gyration is 329 Å. This compares well with the observed value of 335 ± 40 Å.

A further direct verification of the consistency of light scattering and small-angle X-ray-scattering data was carried out. These two techniques are complementary, representing a continuous manifestation of the same phenomenon[18]. The angular distribution of scattering of both X-rays and visible radiation is described by the DEBYE equation[26]

$$P(\theta) = \frac{1}{B^2} \sum_i^B \sum_j^B \frac{\sin 2\pi s r_{ij}}{2\pi s r_{ij}} \tag{7}$$

where B is the total number of scattering elements in the particle, r_{ij} is the distance between elements i and j and s has its previous meaning. Due to the difference in the wavelength of radiation used (light scattering: 3000–5000 Å; X-ray scattering: 1–5 Å), the ranges of s covered by the two are different and consequently the resolution of molecular dimensions is also different: above 1000 Å for light scattering; 10–1000 Å

assumed in further calculations to be monodisperse, as a first approximation. One then calculates weight-average sedimentation coefficients of 32.6 S and 17.2 S, which compare well with the experimental values of 34.2 S and 17.6 S.

Continuing the analysis in terms of a zig-zag chain (or random coil of rods), the theoretical value of the intrinsic viscosity $[\eta]$, was calculated for the molecular weight distribution of Fig. 3. According to FLORY[27], for a random coil

$$[\eta] = \frac{6^{3/2}\,\Phi(\overline{R^2_n})^{3/2}}{\overline{M_n}} \qquad (9)$$

where Φ is a universal constant equal to $2.1 \cdot 10^{21}$. Introduction into Eqn. 9 of the number average radius of gyration and molecular weight, given in Table I, results in $[\eta] = 0.43$ dl/g, close to the experimental value of 0.40.

Model III of Fig. 1 is a branched polymer. While no equation is available for the sedimentation coefficient of a branched structure, an approximate calculation can be made using the theory of viscosity of branched polymers of ZIMM AND KILB[4]. Two models discussed by these authors will be treated: a non-draining polymer with branches radiating from a central segment (the "star" structure) and a randomly branched polymer with tri-functional branch units.

In the first case, ZIMM AND KILB find that the intrinsic viscosities of the branched, $[\eta]_b$, and randomly coiled, $[\eta]_c$, polymers are related to the degree of branching, f, by:

$$g' = [\eta]_b/[\eta]_c = \left(\frac{2}{f}\right)^{3/2} [0.390\,(f-1) + 0.196]/0.586 \qquad (10)$$

In the second case, g' is related to m, the average number of branched units per molecule, by

$$g' = [(1 + m/7)^{1/2} + 4m/9\pi]^{-1/4} \qquad (11)$$

Assuming H-RNA to be a branched structure with all branches of equal length at 78 Å, the values of f and m for the two fractions with $\overline{M_w} = 1.9 \cdot 10^6$ and $5.0 \cdot 10^5$ are 58 and 15. This leads to values of g' of 0.245 and 0.503, for the star-shaped molecule, and of 0.55 and 0.71, for the randomly branched model.

For the sedimentation coefficient calculation, the assumption was made that the product $s[\eta]^{1/3}$ will be identical for coiled and branched structures of identical $\bar{v}$ and molecular weight, an assumption which seems to be generally in use[27,28]. Then, the sedimentation coefficient of the branched structure, s_b, is given by

$$s_b = s_c([\eta]_c/[\eta]_b)^{1/3} \qquad (12)$$

where s_c is the sedimentation coefficient of the randomly coiled structure. Using the KIRKWOOD–RISEMAN equation[29], s_c values of 30.5 S and 16.6 S were calculated for the two main fractions of the RNA. Substitution into Eqn. 12 of these values of s_c together with the above values of g' results in 48.8 S and 20.9 S for the sedimentation coefficients of the two main fractions of the RNA, if the branched molecule is star shaped, and of 37.2 and 18.7 S, for the randomly branched model, which compares reasonably with the experimental values.

While it is difficult to account for the rod-like breakdown product of H-RNA in terms of the degradation of a continuous worm-like chain, this model has been

TABLE II

CALCULATED HYDRODYNAMIC PARAMETERS OF H-RNA

	Slow fraction	*Rapid fraction*	*Total*
$\overline{s_w}$, zig-zag chain	17.2	32.6	26.9
$\overline{s_w}$, branched polymer, "star"	20.9	48.8	38.4
$\overline{s_w}$, branched polymer, random trifunctional	18.7	37.2	30.3
$\overline{s_w}$, wormlike chain	14.3	29.3	23.8
$\overline{s_w}$, experimental	17.6	34.2	28.1
$[\eta]$, zig-zag chain	—	—	0.43
$[\eta]$, experimental	—	—	0.40

cule in solution. This figure, furthermore, shows to scale the amount of detail observable at a resolution of 300 Å in the small-angle X-ray-scattering region.

Comparison of the hypochromic and optical rotatory properties of synthetic polyribonucleotides with H-RNA's from various sources[31-36,45] has been interpreted as an indication that H-RNA contains an amount of WATSON–CRICK double helix[36,45] similar to that deducible from the small-angle X-ray-scattering measurements. Base

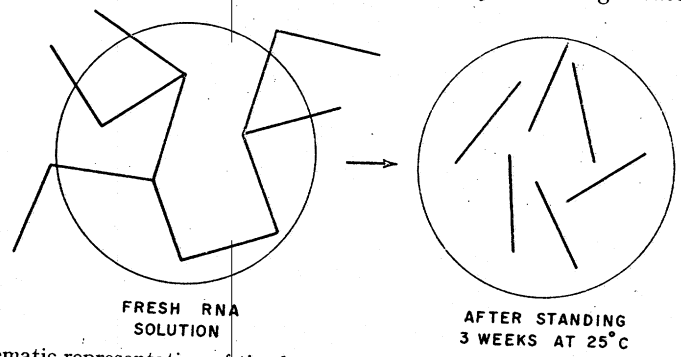

Fig. 6. Schematic representation of the degradation of the H-RNA array of rods. The circles have a diameter of 300 Å, and correspond to the degree of resolution of the small-angle X-ray-scattering experiments.

analysis of Ehrlich ascites tumor cell H-RNA, carried out by BROWN and co-workers[37], permits a degree of base pairing as high as 91 %. This is sufficient to account for the experimental values of 85–90 %, especially when supplemented by occasional pairings of the types described by DONOHUE AND TRUEBLOOD[38]. Small-angle X-ray-scattering observations similar to those described here have been made also on *E. coli* RNA[13]. In that RNA the amount of base pairing theoretically possible is 87–89 %[39,40].

In conclusion, the present analysis has resulted in consistency between hydrodynamic and electromagnetic scattering data when a detailed structure of H-RNA based on the rigid-rodlet substructure was used. The present analysis of hydrodynamic and electromagnetic scattering data, however, has not led to the assignment of a unique structure to H-RNA in terms of a single hydrodynamic model, although the rodlet substructure is highly probable. It is very likely that the actual overall structure (mutual conformation of the rods) does not correspond to any of the simple models

shown recently also to be consistent[46] with the observed angular dependence of the small-angle-ray scattering data and should, therefore, also be examined. For this structure it is necessary to know the persistence length, p. For large values of χ/p, this can be obtained from the radius of gyration, R, according to[1]

$$R^2 = \frac{p^2\chi}{3} \tag{13}$$

where $\chi = L/p$ and L is the contour length of the chain. Using a mass per unit length of 210, the two main "components" of RNA have values of L of $9.05 \cdot 10^3$ Å and $2.4 \cdot 10^3$ Å, which results in $p = 38.5$ and 38 Å, respectively, and $\chi = 235$ and 63. For the case of the present system, the sedimentation coefficient of a wormlike chain has been shown by HEARST AND STOCKMAYER[3] to be given by

$$s = \frac{m_0(1-\bar{v}\rho)}{\varphi_0}\left\{1 + (a/b)\left[\ln\frac{1}{\lambda} - 2.431 + 1.843\,(\lambda N)^{1/2} + 0.138\,(\lambda N)^{-1/2} - 0.305\,(\lambda N)^{-1}\right]\right\} \tag{14}$$

where $1/2\lambda$ is the persistence length in units of b, $N = L/b$ and the other symbols have their meaning of Eqn. 8. Taking the elements, b, as cylinders with a radius equal to that of the rodlets, 11.3 Å, and a length equal to half the persistence length, 19 Å, or about one-fourth that of the rodlets, results in $N = 476$ and 126 for the heavy and light fractions, respectively, $1/\lambda = 4$, $m_0 = 6.64 \cdot 10^{-21}$ g, and $a = 18.8$ Å. The frictional coefficient, φ_0, of the cylindrical element was calculated by the method of HALTNER AND ZIMM[30], *i.e.*, it was taken to be equal to the frictional coefficient of an ellipsoid of equal length and axial ratio (YANG[22]). Use of the PERRIN equation[31] gave $\varphi_0 = 1.81 \cdot 10^{-8}$ dyne·sec·cm. Substitution of these parameters into Eqn. 14 results in sedimentation coefficients of 29.3 S and 14.3 S, or significantly lower than the experimental values.

One further model, that of a swollen hydrated sphere, was examined. This could be formed by cross-linking a random array of short rods. It was found that a degree of hydration of 11 g water per g RNA would be necessary to account for the s values in terms of this model, clearly ruling it out of consideration.

DISCUSSION

The calculated sedimentation coefficients of the two main fractions of ascites tumor cell H-RNA at pH 7 ($I = 0.15$) are compared with the experimental values in Table II. Of the specific models examined, those of the zig-zag chain and the trifunctional branched polymer are in closest agreement with the experimental value. While the experimental data indicate that the rodlets account for at least 90 % of the mass of RNA[32], the present analysis was carried out on the basis of 100 %. Examination of the equation used in the present calculations, *e.g.*, Eqns. 5 and 8, reveals that the error introduced by this degree of uncertainty is small, and certainly well below the differences between parameters obtained for the various structures. It would seem then that, from the point of view of geometric structure, the observed degradation[11,13,33] can be described best by events such as those depicted on Fig. 6, *i.e.*, by the destruction of the flexible joints between the rods leaving the intact rodlike portions of the mole-

treated here, but is probably a complicated hybrid of several such structures, most probably of Models I and III of Fig. 1. The implication is rather strong, however, that the organized double-helical regions observed in RNA gels[41], RNA–protein mixtures[42] and in ribosomes[43] persist when the RNA is dispersed in solution in 0.15 ionic strength at pH 7. The deduction of a unique detailed overall structure, *i.e.*, of the arrangement of the rods to form the large molecule, however, must await the availability of more experimental data, as well as further extension of macromolecular theory.

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